Design, Synthesis, Biological Evaluation And 3D-QSAR Studies Of Novel Benzoxazole Bearing Azetidinone Derivatives

Mansi L. Patil[1], Dr. N.J. Gaikwad[2]
[1]M Pharma, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur -MS, (India)
[2]Professor, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur-MS, India
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Amravati Road, 440 033 Nagpur-MS, India

Abstract
The present study involves synthesis of novel series of Benzoxazole moiety bearing azetidinone 1-(benzo[*d*]oxazol-2-yl)-3-chloro-4-(3-substitutedphenyl) azetidine-2-one derivatives and evaluation of anticancer and anti-inflammatory activity. All the final derivatives were assigned on the basis of IR, ^{1}H NMR and mass spectra and elemental analysis. Electron withdrawing substituents containing chloro and nitro group at *o* and *p* position exhibit good anti-inflammatory and anticancer activity. The 3D-QSAR studies revealed that addition of bulky groups at the phenyl ring will contribute to increase in anti-inflammatory and anticancer activity.

Keywords: Benzoxazole nucleus, Anti-cancer activity, Anti-inflammatory activity.

Introduction

Benzoxazoles are used primarily in industry and being a heterocyclic compound, benzoxazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures[1]. Benzoxazoles have the structural isosters of natural nucleotides adenine and guanine that allow them to easily interact with the biopolymers of living systems[2]. Benzoxazoles are widely used in industry, such as photo stable highly efficient UV dyes, chemosensors chromophores[3,4]. The analogues of Benzoxazole have been entered in the synthesis of new classes of antibacterial drug that showed activity against bacterial infections[5]. Benzoxazole display diverse pharmacological properties including antimicrobial[6], multidrug resistant cancer cell activities[7,8], anti-inflammatory[9,10], antifungal[11], anticonvulsant[12], Antihypertensive[13] Antiviral[14] Antioxidant[15],Antidiabetic[16,17,18], Antitubercular[19] activities . Despite numerous attempts to search and develop new structural prototype as effective cytotoxic agent benzoxazole still remains as potential class of compounds which have been shown to exhibit *in vitro* cytotoxic activity. Even though it shows cytotoxic activity against malignant cells it is found to be less toxic to normal cells. Hence there was need to discover novel benzoxazole analogues as a cytotoxic agent with spectrum of activity that differs from current agents[20] .Benzoxazoles are an important class of heterocyclic that are encountered in a number of natural products. The benzoxazole core structure is found in a variety of cytotoxic natural products, such as Pseudopterazole, UK-1, AJI9561 and Salvianen as well as in biologically active herbicide e.g. Fenoxaprop and in non-steroidal anti-inflammatory drug benoxaprofen.

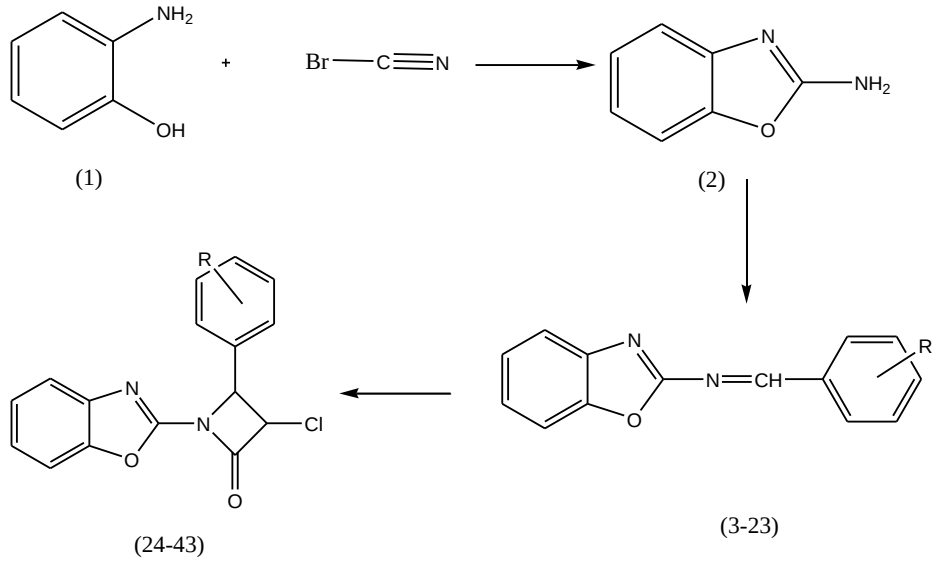

Table 1 list of substituent

Compound No.		-R	Compound No.		-R
(4)	**(24)**	H-	**(14)**	**(34)**	2-OCH_3-
(5)	**(25)**	4-Cl-	**(15)**	**(35)**	4-OCH_3-
(6)	**(26)**	2-NO_2 -	**(16)**	**(36)**	2-Cl-
(7)	**(27)**	4-OH-	**(17)**	**(37)**	3-Br-
(8)	**(28)**	4-OH -3OCH_3-	**(18)**	**(38)**	4-NO_2-
(9)	**(29)**	4-$(CH_3)_2$ -	**(19)**	**(39)**	2,3-$(OCH_3)_2$-
(10)	**(30)**	3-NO_2-	**(20)**	**(40)**	3-Cl-
(11)	**(31)**	3,4,5-$OCH_3)_3$-	**(21)**	**(41)**	-$C_{14}H_{8}$-
(12)	**(32)**	2,4-$(Cl)_2$-	**(22)**	**(42)**	-2,4-$(OCH_3)_2$-
(13)	**(33)**	4-CH_3-	**(23)**	**(43)**	4 C_6H_5-CH_2-O-

Experimental work

Chemistry

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA), S D Fine-Chem (Mumbai, MH, India) and Merck (Darmstadt, Germany), unless specified. Melting points (m.p.) of the synthesized compounds were detected using ThermoNik precision melting point cum boiling point apparatus (model C-PMB-2, Mumbai, MH, India) and are uncorrected. Infrared (IR) spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Tokyo, Japan) at the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharai (RTM) Nagpur University. Proton (1H) and carbon 13 (13C) nuclear magnetic resonance (NMR) were obtained using a BrukerAvance II 400 MHz spectrometer (Billerica, MA, USA), using tetramethylsilane (TMS) as internal standard. The purity of compounds was controlled by thin layer chromatography (silica gel HF254e361, type 60, 0.25 mm; Merck, Darmstadt, Germany). Electrospray ionization mass spectrometry (ESI-MS) was recorded at Waters Q-TOF spectrometer (Waters, Milford, MA, USA) at the Sophisticated Analytical Instrumentation Facility (SAIF), Punjab University (Chandigarh, PB, India).

Synthesis of 2-aminobenzoxazole (2)

Cyanogen bromide 10.59 g (0.1mol) was stirred in methanol/water (7:3; 100 mL), to this a solution of 2-aminophenol 10.91 g (0.1mol) dissolved in a minimum amount of methanol was gradually added with a time lag of 5 min and the mixture was stirred for 40 min. The solution was subsequently neutralized with 1 N sodium hydroxide solution and most of the methanol was removed by distillation. The residue was treated with water and the crude

material was collected by filtration. The obtained product was recrystallized in the presence of charcoal in ethanol to yield **2** (9.26 g) as pure product.
Yield: 75%. mp: 120-122°C (ethanol). R*f*: 0.5 (Toluene:Methanol; 2:8). IR(KBr) cm-1:3378.1, 3217.2 N-H, 1596.21 (C=N), 1601.1 (C=C), 1H NMR (DMSO-d6, 400 MHz) δ: 6.84-6.97 (s, 2H, NH2), 7.10-7.37 (m, 4H, Ar), m/z 134.048 (M+H)+. Anal. calc. for C7H6N2O: C, 62.68; H, 4.51; N, 20.88; O, 11.93.

General Synthesis of N- substituted benzylidinebenzo[d]oxazol-2-amine (3-23)
Compound (**2)** (1.34g g, 0.01 mol) and substituted aromatic aldehydes (**3-23**) (0.01mol) were dissolved in absolute ethanol 25ml, 2-3 drops sulphuric acid was added. Mixture was refluxed on water bath for 8 hrs at 200°C till the completion of reaction (monitored by TLC). The reaction mixture was cooled and poured onto crushed ice to obtain solid which was recrystallized from ethanol to get pure product.

General synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(3-substitutedphenyl)azetidine-2-one derivatives (24-43)
A mixture of compounds (**24-43**) (0.01mol) and triethylamine(0.02mol) was dissolved in 1,4 dioxan(15ml). to this a solution of chloroacetylchloride (0.02mol) was added in solution with vigorous stirring at room temperature for 20minutes. The reaction mixture was heated under reflux for 3 hrs. and the contents were kept at room temperature for 48hrs and poured into ice-cold water. The reaction mixture was filtered washed several times with water and recrystallized from ethanol.

Synthesis of 1-(benzo[d]oxazol-2-yl)3-chloro-4-phenylazetidin-2-one (24)
Yield: 63 %. mp: 223-225 ºC. R*f*: 0.38 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3500.19 (N-H), 1629.43 (C=O), 1324.17 (C-N) 1601.18 (C=N), 2915.76 (C-H), 819.27 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.01-7.56 (s, Ar-H) 4.16-4.93 (s, CH-Cl), 8.00-8.89 (s, CH-N), , EI-MS: m/z $[M+H]^+$ 298. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 64.33; H, 3.71; N, 9.38.

Synthesis of 1-(benzo[d]oxazol-2-yl)3-chloro-4-(4-chlorophenyl)azetidin-2-one (25)
Yield: 74 %. mp: 246-248 ºC. R*f*: 0.44 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3500.19 (N-H), 1629.43 (C=O), 1324.17 (C-N) 1601.18 (C=N), 2915.76 (C-H), 819.27 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.10-7.56 (s, Ar-H), 4.16-4.93 (d, J=4 Hz, 1H, CH-Cl), 7.01(d, J=8Hz, Ar-CH), EI-MS: m/z $[M+H]^+$ 332.01. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 57.68; H, 3.03; N, 8.41.

Synthesis of 1-(benzo[d]oxazol-2-yl)3-chloro-4-(2-nitrophenyl)azetidin-2-one (26)

Yield: 70 %. mp: 256-258 ºC. R*f*: 0.49 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3549.73 (N-H), 1696.81 (C=O), 1299.67 (C-N) 1652.64 (C=N), 2957.68 (C-H), 884.10 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.20-7.85 (s, 8H, Ar-H), 4.53-5.90 (d, J=4 Hz, CH-Cl), 6.98 (d, J= 8 Hz, Ar-CH), EI-MS: m/z $[M+H]^+$ 343.04. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 55.19; H, 2.93; N, 12.13.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(4-hydroxyphenyl)azetidin-2-one (27)
Yield: 62 %. mp: 244-246 ºC. R*f*: 0.46 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3370.42 (N-H), 1692.09 (C=O), 1280.63 (C-N) 1653.27 (C=N), 2985.14 (C-H), 857.47 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 4.54-4.55 (s, CH-Cl), 8.01-8.07 (s, CH-N), 6.90-7.84 (m, 8H, Ar-H), 4.54-4.55 (d, J=4 Hz, CH-Cl) , 6.88 (d, J=8 Hz, Ar-CH), EI-MS: m/z $[M+H]^+$ 314.05. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 61.06; H, 3.52; N, 8.90.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(4-hydroxy-3-methoxyphenyl)azetidin-2-one (28)

Yield: 60 %. mp: 276-278 ºC. R*f*: 0.41 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3327.02 (N-H), 1624.37 (C=O), 1311.42 (C-N) 1693.05 (C=N), 2986.40 (C-H), 802.85 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.05-7.72 (m, 7H, Ar-H) 4.21-4.26 (d, 4.3 Hz, 1H, CH-Cl), 7.06 (d J= 8 Hz, Ar-CH), EI-MS: m/z $[M+H]^+$ 348.37. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 65.63; H, 4.89; N, 20.15..

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(4-dimethylamino)phenyl)azetidin-2-one (29)
Yield: 66 %. mp: 209-211 ºC. R*f*: 0.43 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3306.59 (N-H), 1612.08 (C=O), 1286.34 (C-N) 1673.47 (C=N), 2928.92 (C-H), 817.76 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.13-7.95 (s, 8H, Ar-H), 4.45-4.67 (d, J=4.3Hz, CH-Cl), 6.85(d, J=8.4Hz, 1H, CH-Ar), 3.67 (s, 6H, OCH_3), EI-MS: m/z $[M+H]^+$ 341.09. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 63.25.63; H, 4.72; N, 12.29.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(3nitrophenyl)azetidin-2-one (30)

Yield: 75 %. mp: 251-253 ºC. R_f: 0.44 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3200.73 (N-H), 1645.89 (C=O), 1220.63 (C-N) 1600.95 (C=N), 3100.75 (C-H), 843.16 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.01-7.95 (m, 8H, Ar-H) 3.36 (d, J=4 Hz, 1H, CH-Cl) 7.01-7.95 (d, J=8 Hz, Ar-CH), EI-MS: m/z $[M+H]^+$ 343.04. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 65.63; H, 4.89; N, 20.15. Found: C, 55.91; H, 2.93; N, 12.23.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (31)

Yield: 64 %. mp: 243-245 ºC. R_f: 0.42 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3160.97 (N-H), 1590.43 (C=O), 1296.27 (C-N) 1665.03 (C=N), 3021.53 (C-H), 871.09 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.01-7.95(m, 6H, Ar-H), 4.16-4.17 (d, J=4 Hz, CH-Cl), 6.90-6.99(d, J=8Hz, 1H, CH-Ar), 2.52 (s, 9H, OCH_3), EI-MS: m/z $[M+H]^+$ 388.8. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 58.69; H, 4.41; N, 7.21. Found: C, 65.03; H, 4.79; N, 19.75.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(2,4-dichlorophenyl)azetidin-2-one (32)

Yield: 77 %. mp: 231-233 ºC. R_f: 0.46 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3346.67 (N-H), 1620.79 (C=O), 1308.76 (C-N) 1669.38 (C=N), 3021.03 (C-H), 823.08 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.13-7.84 (m, 7H, Ar-H), 4.91-5.26 (d, J=4.2Hz, 1H, CH-Cl), 6.87(d, J=8.2Hz, 1H, CH-Ar), EI-MS: m/z $[M+H]^+$ 365.97. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 52.58; H, 2.47; N, 7.67. F

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-p-tolyazetidin-2-one (33)

Yield: 63 %. mp: 251-253 ºC. R_f: 0.42 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3412.26 (N-H), 1602.73 (C=O), 1280.28 (C-N) 1683.18 (C=N), 3320.19 (C-H), 846.78 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.19-7.63 (m, Ar-H)5.06 (s, CH-Cl), 8.17-8.73 (s, CH-N),), EI-MS: m/z $[M+H]^+$ 312.07. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 65.29; H, 4.19; N, 8.96.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(2methoxyphenyl)azetidin-2-one (34)

Yield: 62 %. mp: 276-278 ºC. R_f: 0.38 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3400.15 (N-H), 1690.87 (C=O), 2920.09 (C-N) 1620.69 (C=N), 2900.4 (C-H), 820.78 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 6.58-7.69 (s, Ar-H), 5.56 (d, J=4.3Hz, 1H, CH-Cl), 6.55 (d, J=8.3Hz, 1H, Ar-CH), EI-MS: m/z $[M+H]^+$ 328.06. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 62.11; H, 3.99; N, 8.52.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(4-methoxyphenyl)azetidin-2-one (35)

Yield: 67 %. mp: 237-239 ºC. R_f: 0.41 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3396.73 (N-H), 1686.11 (C=O), 1373.18 (C-N) 1658.34 (C=N), 2915.49 (C-H), 846.78 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 6.67-7.43 (m, 8H, Ar-H), 4.69 (d, J= 4Hz, 1H, CH-Cl), 7.16 (d, J=8.1Hz, 1H, CH-Ar), EI-MS: m/z $[M+H]^+$ 328.06. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 62.11; H, 3.99; N, 8.52. Found: C, 65.03; H, 4.79; N, 19.75.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(2-chlorophenyl)azetidin-2-one (36)

Yield: 71 %. mp: 259-261 ºC. R_f: 0.44 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3400.46 (N-H), 1649.14 (C=O), 1289.45 (C-N) 1648.73 (C=N), 2873.27 (C-H), 818.70 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.19-7.93 (m, 8H, Ar-H), 5.08 (d, J=4Hz, 1H, CH-Cl) 6.73 (d, J=8.4Hz, 1H, CH-Ar), EI-MS: m/z $[M+H]^+$ 332.01. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 57.68; H, 3.03; N, 8.41.

Synthesis of 1-(benzo[d]oxazol-2-yl)-4-(3-bromophenyl)-3-chloroazetidin-2-one (37)

Yield: 76 %. mp: 266-268 ºC. R_f: 0.47 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3398.46 (N-H), 1649.93 (C=O), 1308.72 (C-N) 1600.51 (C=N), 2987.37 (C-H), 814.67 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.21-7.76 (m, 8H, Ar-CH), 4.86-4.94 (d, J=4.3Hz, 1H, CH-Cl), 6.22 (d, J=8Hz, 1H, CH-Ar) EI-MS: m/z $[M+H]^+$ 377.96. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 50.89; H, 2.67; N, 7.42.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-choloro-4-(4-nitrophenyl)azetidin-2-one (38)

Yield: 74 %. mp: 270-272 ºC. R_f: 0.39 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3428.75 (N-H), 1602.29 (C=O), 1296.47 (C-N) 1606.19 (C=N), 2971.69 (C-H), 849.26 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.09-7.48 (m, 8H, Ar-H) 4.76 (d, J=4.2Hz, 1H, CH-Cl), 6.48(d, J=8.2Hz, 1H, CH-Ar), EI-MS: m/z $[M+H]^+$ 343.04. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 55.9; H, 4.93; N, 12.23

Synthesis of 1-(benzo[d]oxazol-2-yl)-4-(2,3 dimethoxyphenyl)-3-chloroazetidin-2-one (39)

Yield: 63 %. mp: 217-219 ºC. R_f: 0.39 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3400.63 (N-H), 1635.17 (C=O), 1308.27 (C-N) 1600.19 (C=N), 2987.69 (C-H), 879.14 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.23-7.51 (m, 7H, Ar-H), 4.67-4.82 (d, J=4.2Hz, 1H, CH-Cl), 6.86 (d, J=8.1Hz, 1H, CH-Ar), EI-MS: m/z $[M+H]^+$ 343.04. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 55.9; H, 2.93; N, 12.23.

Synthesis of 1-(benzo[d]oxazol-2-yl)-4-(3-chlorophenyl)-3-chloroazetidin-2-one (40)

Yield: 76 %. mp: 276-278 ºC. R_f: 0.46 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3458.13 (N-H), 1605.19 (C=O), 1345.29 (C-N) 1653.28 (C=N), 2955.76 (C-H), 822.14 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 6.76-7.82 (m, 8H, Ar-H), 4.22-4.63 (d, J=4.2Hz, CH-Cl), 6.74(d, J=8.2Hz, 1H, Ar-CH), EI-MS: m/z $[M+H]^+$ 358.07. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 60.26; H, 4.21; N, 7.81.

Synthesis of 1-(benzo[d]oxazol-2-yl)-4-(2,4 dimethoxyphenyl)-3-chloroazetidin-2-one (41)

Yield: 64 %. mp: 208-210 ºC. R_f: 0.38 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3496.63 (N-H), 1621.76 (C=O), 1300.97 (C-N) 1673.56 (C=N), 2920.92 (C-H), 807.85 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 7.25-786 (m, 7H, Ar-H), 4.15-4.29 (d, J=4.2Hz, CH-Cl), 6.86 (d, J=8.2Hz, 1H, Ar-CH), EI-MS: m/z $[M+H]^+$ 358.07. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 60.26; H, 4.21; N, 7.81.

Synthesis of 1-(benzo[d]oxazol-2-yl)-3-chloro-4-(2-(furan-2-yl)phenyl)azetidin-2-one (42)

Yield: 60 %. mp: 276-278 ºC. R_f: 0.49 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3458.13 (N-H), 1605.19 (C=O), 1345.29 (C-N) 1653.28 (C=N), 2955.76 (C-H), 822.14 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 6.76-7.82 (m, 8H, Ar-H), 4.22-4.63 (d, J=4.2Hz, CH-Cl), 6.74(d, J=8.2Hz, 1H, Ar-CH), EI-MS: m/z $[M+H]^+$ 288.03. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 58.25; H, 3.14; N, 9.70.

Synthesis of 1-(benzo[d]oxazol-2-yl)-4-(2,4benzyloxyoxyphenyl)-3-chloroazetidin-2-one (43)

Yield: 62 %. mp: 234-236 ºC. R_f: 0.41 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 3376.14 (N-H), 1367.90 (C=O), 1317.06 (C-N) 1693.22 (C=N), 2974.84 (C-H), 898.23 (C-Cl) , ^{1}H NMR (DMSO, ppm): δ 723-7.76 (m, 13H, Ar-CH), 4.64-4.73 (d, J=4.2Hz, 1H, CH-Cl), 6.78(d, J=8.3Hz, 1H, Ar-CH), EI-MS: m/z $[M+H]^+$ 404.09. Anal. Calcd for $C_{19}H_{17}N_5O_2$: C, 68.23; H, 4.23; N, 6.92.

Pharmacology

The experimental protocols for the pharmacological screening on mice were done with Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj (RTM) Nagpur University, Nagpur, India

Animals

The preferred rodent species that was albino mice of either sex were used. Each animal, at the commencement of its dosing, was of 8-12 weeks old. The temperature in the experimental animal room was 22^0 ($\pm$ 3^0). The relative humidity was maintained between 30-70%. The conventional laboratory animal diet was used for feeding and unlimited drinking water was supplied. Animals were grouped in the cages.

Anti-inflammatory activity

Anti-inflammatory activity of all synthesized compounds were quantified, *in vivo*, by carrageenan induced rat paw edema method using digital vernier calliper. All the test compounds were suspended in 0.5 % of CMC and administered orally. The Albino wistar rats were treated orally with the newly synthesized derivatives (100 mg/kg, p.o.) and standard drug indomethacin (10 mg/kg, i.p.), 1 h prior to the 1% (w/v) solution injection of 0.1 ml carrageenan into plantar region of right hind paw (subcutaneously). The relative paw thickness was measured at an interval of 0 h, 1 h, 2 h, 3 h in the individual animal of the control, test and the standard group **(Table 2)**. The percent inhibition of paw edema was calculated using following formula,

$$\% \text{ Inhibition } (\%I) = [1- (D_t/D_c)] \times 100$$

Where,
Dt = Mean relative change in paw thickness in test group/standard group.
Dc = Mean relative change in paw thickness in control group.

table 2

Sr.No	Group	Dose mg/kg (body wt.of rats)	% inhibition of paw edema			
1.		10mg/kg	0h	1 h	2h	3h
2.	Control	100mg/kg	4.12 ±0.75	4.39 ±0.14	4.27 ±0.18	4.16 ±0.28
3.	Indomethacin	100mg/kg	1.48 ±0.16	1.29 ±0.28	1.33 ±0.22	1.410.11
4.	24	100mg/kg	3.27 ±0.49	3.16 ±0.59	3.05 ±0.13	3.08 ±0.37
5.	25	100mg/kg	2.65 ±1.19	2.77 ±0.15	2.67 ±0.46	3.03 ±0.46
6.	26	100mg/kg	2.98 ±0.58	3.12 ±0.66	3.4 ±0.75	3.7 ±0.73
7.	27	100mg/kg	3.82 ±0.29	3.7 ±0.12	4.09 ±0.24	3.91 ±0.67
8.	28	100mg/kg	3.78 ±0.64	4.14 ±0.69	4.18 ±0.38	3.36 ±0.21
9.	29	100mg/kg	3.99 ±1.08	3.6 ±0.27	3.3 ±0.56	3.03 ±0.48
10.	30	100mg/kg	2.88 ±1.05	2.92 ±0.94	2.74 ±0.71	2.78 ±0.35
11.	31	100mg/kg	3.38 ±0.83	3.34 ±0.11	3.28 ±0.29	3.16 ±0.32
12.	32	100mg/kg	3.04 ±0.42	2.68 ±0.85	3.18 ±0.40	2.97 ±0.24
13.	33	100mg/kg	4.56 ±0.71	4.62 ±0.27	4.19 ±0.83	4.18 ±0.27
14.	34	100mg/kg	4.72 ±0.14	4.25 ±0.46	4.26 ±0.16	4.46 ±0.92
15.	35	100mg/kg	3.81 ±0.36	3.62 ±0.27	3.39 ±0.19	3.91 ±0.54
16.	36	100mg/kg	3.29 ±0.51	3.48 ±0.15	3.51 ±0.25	3.08 ±0.29
17.	37	100mg/kg	3.76 ±0.26	3.51 ±0.46	3.57 ±0.37	3.85 ±0.76
18.	38	100mg/kg	2.84 ±0.11	2.81 ±0.28	2. 72 ±0.86	2.63 ±0.48
19.	39	100mg/kg	3.15 ±0.19	3.3 ±0.54	3.41 ±0.64	3.46 ±0.14
20.	40	100mg/kg	3.12 ±0.88	3.46 ±0.23	3.29 ±0.14	2.96 ±0.23
21.	41	100mg/kg	4.26 ±0.47	4.12 ±0.17	4.13 ±0.68	1.41±0.82
22.	42	100mg/kg	3.81 ±0.16	3.62 ±0.26	3.64 ±0.12	3.73 ±0.17
23.	43	100mg/kg	3.98 ±0.47	3.81 ±0.44	4.02 ±0.86	3.91 ±0.14

Cytotoxic Screening of Scheme I Compounds

Onion root model was used for studying cytotoxic activity. Allium cepa root tip meristems have been widely used for the evaluation of cytotoxic and antimitotic activity. The inhibitory effect of synthesized compounds was evaluated on the growth of *allium cepa* root meristems and the effect was compared with standard anticancer drug cyclophosphamide.

4.3.1 Growing *Allium cepa* meristems

Locally available *allium cepa* bulbs were grown in dark over 100 mL tap water at ambient temperature until the roots have grown to approximately 3-4 cm. The water was changed daily.

4.3.2 Conditions for drug incubation

Working dilutions of all the drugs were made in tap water. Standard drug cyclophosphamide and compound **(24-43)** were used 10 mg/mL concentration. The bulbs with root tips grown up to 3-4 cm were placed over drug solution and incubation was carried out at ambient temperature.

Table 3: Root length attained after incubation

Groups	Root length after hour[#]				
	0 h	24 h	48 h	72 h	96 h
Control	3.92±0.25	4.83±0.17	4.92±0.48	4.95±0.37	4.81±0.24
24	3.90±0.56	4.22±0.29	4.01±0.48	4.33±0.23	4.04±0.11
25	4.01±0.86	3.60±0.08	3.50±0.32	3.51±0.67	3.42±0.75
26	4.21±0.28	4.13±0.13	4.02±0.09	4.25±0.15	4.31±0.78
27	4.02±0.12	4.11±0.58	4.17±0.14	4.16±0.06	4.25±0.19
28	3.90±0.65	3.92±0.34	4.05±0.25	4.18±	4.02±0.15
29	4.12±0.22	4.05±0.21	4.12±0.48	4.20±0.54	4.32±0.67
30	4.05±0.16	3.71±0.28	3.64±0.66	3.50±0.07	3.40±0.67
31	3.90±0.15	4.01±0.04	4.11±0.18	4.21±0.29	4.23±0.11
32	4.25±0.06	4.17±0.29	4.22±0.44	4.26±0.73	4.37±0.18
33	4.02±0.27	4.14±0.16	4.05±0.07	4.21±0.29	4.20±0.46
34	3.90±0.09	3.94±0.15	4.11±0.46	4.23±0.38	4.37±0.82
35	3.92±0.13	3.94±0.08	3.90±0.74	4.02±0.29	4.17±0.05
36	3.90±0.68	3.84±0.27	3.70±0.23	3.71±0.06	3.62±0.01
37	4.02±0.02	3.81±0.76	3.74±0.21	3.72±0.40	3.58±0.81
38	4.12±0.20	3.71±0.47	3.60±0.36	3.51±0.08	3.42±0.77
39	4.12±0.12	4.22±0.76	4.30±0.40	4.44±0.35	4.42±0.63
40	3.97±0.08	3.75±0.50	3.63±0.68	3.66±0.15	3.68±0.27
41	4.06±0.23	4.24±0.56	4.25±0.81	4.36±0.06	4.41±0.70
42	4.19±0.12	4.15±0.57	4.27±0.69	4.36±0.48	4.41±0.37
43	4.15±0.30	4.08±0.16	4.63±0.11	4.27±0.85	4.30±0.16
Standard	3.94±0.13	2.96±0.85	2.98±0.74	2.96±0.04	2.45±0.13

3D-QSAR Study

The 3D-QSAR was performed using the molecular modeling software package VLife Molecular Design Suite (VLifeMDS) version 4.3.1 on HP-PC (HPLV1911) with a Pentium IVprocessor and Windows 7 operating system

Methodology

The Modules >>QSARPlus>> 3D-QSAR from the main menu of MDS was selected to launch the worksheet. By default all the molecules in a directory were considered for QSAR. QSAR tool was chosen from which molecules were opened, the subfolder containing set of molecules was selected. Activity data which was stored as 'activity.txt' was inserted by selecting File >> Insert Data. The field parameters electrostatic, hydrophobic and steric were computed by selecting QSAR Tools >> Compute Field window. The Gasteiger-Marsili charge was selected for the computation and invariable columns were removed.

The data selection was done by choosing QSAR Tools >> Data Selection. Training data set selection method was applied to create training and test set for this random selection was done. The data was selected in the range of 65% to 85%. Finally, Variable Selection and Model Building Wizard tool was selected for the application of statistical methods like kNN, PLSR, MLR and PCR from Advanced Methods >> Method. The statistical data was generated which results in coefficient of determination (r^2), cross validated coefficient of determination (q^2), r^2 for external test set (pred_r^2) fitness plot and points of distribution.

Results and discussion

Chemistry

The reported investigation deals with synthesis and characterization of several benzoxazole bearing azetidinone nucleus to form final twenty derivatives. a new series of 2-substituted benzoxazole schiff bases and its azetidinone 1-(benzo[*d*]oxazol-2-yl)-3-chloro-4-(3-substitutedphenyl)azetidine-2-one derivatives. were synthesized from o-amino phenol and cyanogen bromide and It is cycloaddition reaction in which a catalytic reagent cyanogen bromide act as a nucleophile and by reacting with o-aminophenol and forms 2-aminobenzoxazole The IR spectra of compound **(2)** showed the presence of characteristic absorption peak at 3356.14 cm^{-1} of N-H and 1687.21 cm^{-1} of C=O can be seen in the **Figure 17**. The ^{1}H NMR spectra of compound **(2)** showed two protons at δ 10.57 for NH_2 and the protons belonging to the aromatic part showed presence in the range of δ 7.10-7.37 for four protons. 2-aminobenzoxazole again reacts with substituted benzaldehydes to form schiff bases intermediates **(4-23)** showed C=N, C=C, C-H stretching bands in the region 3609.79-1696.76 cm^{-1}, 1501.67-1596.19 and 2876.53-3064.28 respectively in their IR spectrum. While in their ^{1}H NMR spectra for these compounds exhibited singlet for C-H in the regions of δ 8.01-8.73 and δ 2.81-3.56 (OCH_3) and multiplet in the region of δ 7.01-7.86 (Ar-H). Followed by formation of azetidinone derivatives. **(24-43).** IR spectra of compounds showed bands of various functional groups present, C=O stretching in the range 1713.69-1792.61 cm^{-1}, C=N stretching in the range 1600.19-1694.75 cm^{-1}, C-H stretching 2873.27-2993.26 cm^{-1} and C-N stretching 1220.63-1373.18 cm^{-1}. The ^{1}H NMR spectra of these compounds exhibited multiplet around δ 6.76-7.95 (Ar-H). It also exhibited the characteristic signals for protons as doublet around δ 4.16-5.90, while the methoxy protons showed singlet in the region of δ 2.52-3.67 and singlet at δ 10.74 for (OH).

Pharmacological screening

The results of anti-inflammatory activity revealed that the compounds (**25, 30, 32, 38**) showed significant activity standard drug Indomethacin was used while compounds (**36, 37, 39**) showed moderate activity, compounds **(33, 34)** showed poor anti-inflammatory activity**.** Carrageenan induced paw oedema method was used for the studies. The pharmacological screening of compounds for anticancer activity was done using onion root model. Compounds **25, 30** and **38** were found to have good cytotoxic activity, whole compounds **36,37** showed moderate activity and compounds **39, 42** exhibited poor cytotoxic activity.

3D-QSAR Study

The results obtained from anti-inflammatory activity were statistically analyzed by 3D QSAR model and found that the multiple linear regression (kNN-MLR) method for 3D QSAR gave the best of results. The values of r^2=0.857, q^2=0.8021, F-test=10.0050, Pred_r^2= 0.4460 and Pred_r^2se=9.3000 were best of the four methods, The fitness plot showed a linear distribution of the test and training set which is supported by the diagrammatic presentation of the training set and the test set analysis by the QSAR method as presented in Figure 1,2 and 3 The contribution plot and the 3D-QSAR graphical interface provides with the points, points generated in the model is E_208 accounting for electrostatic fields at the lattice points on the grid as shown in the figure, this point suggest the significance and requirements electrostatic properties in the structure to maximize the anti-inflammatory activity. From the 3D diagram and coordinates it is observed that the descriptor (E_208) on the positive scale near the phenyl ring indicates the addition of electropositive group to enhance the activity. The actual verses predicted activity

values were mentioned in the Table 4, which represents the residual values, it is found that except in certain cases there is very less significant difference in the actual and predicted activity which provides with good predictive ability of the QSAR model, this can be observed from the fitness plot.

The results obtained from anticancer activity were statistically analyzed by 3D-QSAR model and found that the multiple linear regression (kNN-MLR) method for 3D-QSAR gave the best of results. The values of r^2=0.7579, q^2=0.5822, F-test=21.0024, Pred_r^2= 0.2364 and Pred_r^2se=5.7147 were best of the four methods,. The fitness plot showed a linear distribution of the test and training set which is supported by the diagrammatic presentation of the training set and the test set analysis by the QSAR method as presented in Figure 4, 5 and 6. The contribution plot and the 3D-QSAR graphical interface provides with the points, points generated in the model is S_439 accounting for steric fields at the lattice points on the grid as shown in the figure, these points suggest the significance and requirements of the steric properties in the structure to maximize the anticancer activity. From the 3D diagram and coordinates it is observed that the steric descriptor (S_223) is near the phenyl ring of benzoxazole, it indicates the need of bulky group to maximize the activity. The actual verses predicted activity values were mentioned in the Table 7, which represents the residual values, it is found that except in certain cases there is very less significant difference in the actual and predicted activity which provides with good predictive ability of the QSAR model, this can be observed from the fitness plot

Table 4: data set used for 3d QSAR analysis with actual and predicted activities of anti-inflammatory compounds (24-43)

Compound No.	Actual activity	Predicted activity	Residual	Compound No.	Actual activity	Predicted activity	Residual
24	26.95	36.913255	-9.96325	**34**	22.04	35.660911	-13.6209
25	35..61	38.443388	-2.83338	**35**	31.93	43.510388	-11.5803
26	39.00	33.48256	5.51744	**36**	38.26	28.584636	9.675364
27	28.28	28.461789	-0.18178	**37**	25.36	36.766571	-11.4065
28	33.82	39.184324	-5.36432	**38**	43.16	49.814197	-6.6541
29	35.67	37.862196	-2.19219	**39**	28.44	40.839324	-12.3993
30	49.68	47.239359	2.44065	**40**	32.71	43.885808	-11.1758
31	29.18	40.609048	-11.4290	**41**	24.21	45.302701	-21.0927
32	43..14	47.166472	-40.2647	**42**	27.35	27.220-119	0.12989
33	17.34	30.788826	-13.4488	**43**	34.53	35.660911	-11.3091

Table 5: unicolumn statistics analysis of the training and test sets for 3d QSAR anti-inflammatory model (24-43)

Data Set	Average	Maximum	Minimum	Standard deviation	Sum
Training Set	35.9217	49.1680	22.3650	8.7237	502.90
Test Set	39.0705	47.6890	28.2800	6.9221	234.4230

Table 6: statistical results of 3d-QSAR KNN-MLR model generated by stepwise variable selection method

1.	N	14
2.	Degree of freedom	12
3.	r2	0.8547
4.	q2	0.8201
5.	F_test	10.0050
6.	r2_se	6.7052
7.	q2_se	7.4871
8.	pred_r2	0.5460
9.	pred_r2se	9.3000

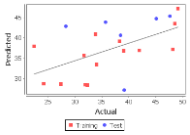

figure 1:comparison of observed activity versus predicted activity for training set & test set compounds according to 3d-QSAR anti-inflammatory model by KNN-MLR method

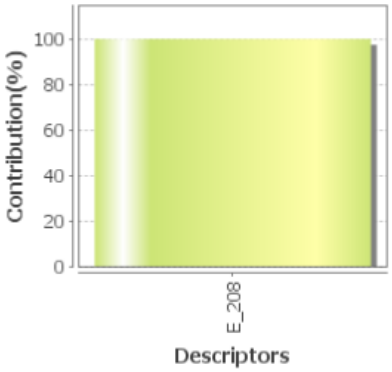

figure 2: contribution plot for steric and electrostatic descriptors selected in 3D-QSAR model by KNN-MLR method

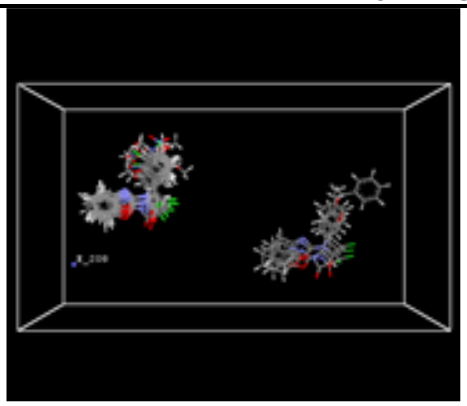

figure 3: stereo view of molecular rectangular field grid around the superposed molecular units of benzoxazole azetidinone derivatives using KNN-MLR method

Table 7: data set used for 3d qsar analysis with actual and predicted activities of anticancer compounds (24-43)

Compound No.	Actual activity	Predicted activity	Residual	Compound No.	Actual activity	Predicted activity	Residual
24	14.81481	17.186685	-2.37187	**34**	14.81481	17.850488	-3.035678
25	21.10288	17.85049	3.25198	**35**	15.22634	19.005797	-3.779457
26	18.93004	16.801603	21.28437	**36**	13.46091	19.28678	-5.825878
27	14.09465	17.850469	-3.75581	**37**	14.93004	19.850428	-4.92038
28	16.87243	16.967727	-0.09529	**38**	20.16049	16.713866	-9.130414
29	18.107	17.314846	0.792154	**39**	10.81481	19.278514	-8.463704
30	21.21811	16.889329	4.328781	**40**	13.04527	17.282399	-4.237129
31	20.78189	18.269415	2.512475	**41**	11.28395	19.273422	-7.989472
32	16.04938	17.027176	-0.97779	**42**	14.87243	28.753148	-13.88071
33	16.46091	17.186685	-0.72577	**43**	17.69547	20.128262	-2.432792

Table 8: unicolumn statistics analysis of the training and test sets for 3D QSAR anticancer model (24-43)

Data Set	Average	Maximum	Minimum	Standard deviation	Sum
Training Set	18.3201	28.8060	14.0904	4.0178	238.16
Test Set	19.1646	27.1600	14.8140	4.9431	134.152

table 5: statistical results of 3d-qsar KNN-MLR model generated by stepwise variable selection method

1.	N	14
2.	Degree of freedom	12
3.	r2	0.7579
4.	q2	0.5822
5.	F_test	21.0024
6.	r2_se	8.2549
7.	q2_se	7.4730
8.	pred_r2	0.5364
9.	pred_r2se	5.7147

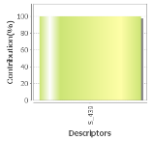

figure 4: contribution plot for steric and electrostatic descriptors selected in 3D-QSAR anticancer model by KNN-MLR method

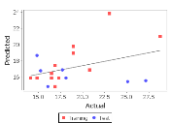

figure 5:comparison of observed activity versus predicted activity for training set & test set compounds according to 3D-QSAR anti-cancer model by KNN-MLR method

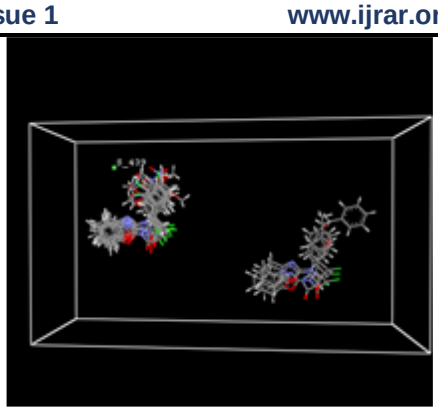

figure 6: stereo view of molecular rectangular field grid around the superposed molecular units of benzoxazole azetidinone derivatives using KNN-MLR method

Conclusion

In summary, we have described the synthesis of novel benzoxazole bearing azetidinone derivatives by conventional method with high purity and better yields of product. All the spectral studies were in good agreement with the final structures of the derivatives. All synthesized compounds were evaluated for anti-inflammatory and anticancer activity. Compounds 25,30,32,38 exhibiting promising anti-inflammatory activity while compounds 25, 30 and 38 exhibited good anticancer activity.. The 3D-QSAR study results revealed addition of electropositive group to enhance the anti-inflammatory activity while it indicated need of bulky group to enhance anticancer activity. Hence, these studies are useful in understanding the structural requirements for design of novel and potent anti-inflammatory and anticancer molecules.

Acknowledgement

We are thankful to University Department of Pharmaceutical Sciences, Nagpur University for providing us good working environment with resources to carry out research work.

References

1. Vinsova J, Horak V, Buchta V, Kaustova J. 2005. Highly Lipophilic Benzoxazoles with Potential Antibacterial Activity. Molecules, 10(2): 783-793.
2. Sondhi SM, Singh N, Kumar A, Lozach O and Meijer L. 2006. Synthesis anti-inflammatory analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/ benzoxazole derivatives and some Schiff's bases. Bioorg. & Med. Chem. 7(3): 3758–3765.
3. Yildiz-Oren I, Yalcin I, Aki-Sener E, and Ucarturk N. 2004. Synthesis and structure-activity relationships of new antimicrobial active multisubstituted benzazole derivatives. Eur. J. of Med. Chem. 39(5): 291–298.
4. Guzow K, Szmigiel D, Wroblewski D, Milewska M, Karolczak J and Wiczk W. 2007. New fluorescent probes based on 3-(2-benzoxazol-5-yl)alanine skeleton—Synthesis and Photophysical properties. Journal of Photochemistry and Photobiology A: Chemistry. 187(4): 87–96.
5. Um SI. 2007. The synthesis and properties of benzoxazole fluorescent brighteners for application to polyester fibers. Dyes and Pigments. 75(2): 185–188.
6. Ertan T, Yildiz I, Tekiner-Gulbas B, Bolelli K, Arpac OT, Ozkan S, Kayank F. 2009. Synthesis, biological evaluation and 2D-QSAR analysis of benzoxazoles as antimicrobial agents. Eur. J. Med. Chem. 44(1): 501-510.

7. Kumar D, Jacob MR, Reynolds MB, Kerwin SM. 2010. Synthesis and Evaluation of anticancer benzoxazoles and benzimidazoles related to UK—1. Bioorg. & Med. Chem. 10: 3997-4004.
8. Kamal A, Reddy KS, Khan MN, Shetti R, Pushpavalli S, Shriniwas C, Bhadra MP, Chourasia M. 2010. Synthesis DNA binding ability and anticancer activity of benzthiazoles/benzoxazole-prrolo[2-1-c][1,4] benzodiazepine conjugates. Bioorg. & Med. Chem. 18: 4747-4761.
9. Garrepali S, Sarangapani M, Garapalli P, Chilukala S. D. 2011. Design, synthesis and biological evaluation of benzoxazole derivatives as cyclo-oxygenase-2 inhibitors. Der Pharmacia Letters. 3(2): 427-432.
10. Patil ST, Bhatt P. 2010. Synthesis and pharmacological screening of some benzoxazole derivatives as an anti-inflammatory agents. IJPRD. 2: 0974-9446.
11. Kim BJ, Kim YK, Choi Y, Choo HP. 2010. Synthesis of benzoxazole amides as novel antifungal agents. Bull. Korean. Chem. Soc. 31(4): 1270-1274.
12. Siddiqui N, Sarafroz M, Alam M, Ahsan W. 2008. Synthesis anticonvulsant & neurotoxic evaluation of 5-carbomethoxybenzoxazole derivatives. Drug Research. 65(4): 449-445.
13. Bhandari, S, Bothra, KG. 2009. Design, Synthesis and Pharmacological Screening of NovelAntihypertensive Agents using Hybrid Approach. Bioorg. Med. Chem.17: 390-400.
14. Shaoyong K, Yanhong M, W Ziwen, Yang K. Ying Liang W. 2013. Novel Cycloalkylthiophene–imine Derivatives bearing Benzothiazole Scaffold: Synthesis Characterization and Antiviral Activity Evaluation. Bioorg. & Med. Chem. Lett. 23(10): 5131-5134.
15. Ismael Yekini A, Fatma Hammoudi A, Martin-Nizard F, Yous S, Nicolas, Pascal L, Pascal Carato. 2009. Antioxidant activity of benzoxazolinonic and benzothiazolinonic derivatives. Bioorg. & Med. Chem. 17(5): 7823–7830.
16. Mokale SN, Elgire RD, Sakle N, Shinde DB. Synthesis, hypolipidemic & hypoglycemic activity of some 2-(4-(2-substituted aminothiazole-4-yl)phenoxy)-2-methyl propionic acid derivatives. 2011. Bioorg. & Med. Chem. 21: 682-685.
17. Narsimha Reddy RA, Rajyalakshmi G, Kishore Kumar A, Sarangapani M. Antihyperglycemic Activity Of N-(Benzo[D]Oxazol-2-Yl)-2-(5-Bromo-2-Oxoindolin-3-Y Lidene) Hydrazine Carboxamide In Diabetic Chicks.2014. Int. J. Pharmac Res. 4(2):52-54.
18. Reddy RN. Antidiabetic Activity of 2-Amino-(5-Fluoro-2-Oxoindolin-3-Ylidene) Benzoxazole-5-Carbohydrazide In Rats. 2014. J. Of. Biomed and Pharma. Res. 3(5): 78-81.

19. Mustafa A,Temiz-Arpaci O, Kaynak-Onurdag F, Ozgen S. Mycobacterial Properties Of Some 2,5-Disubstituted-Benzoxazole Derivatives. J. Fac. Pharm. 39 (2): 155-162.

20. Patil ST, Bhatt PA. Synthesis and characterization of some benzoxazole derivatives. 2010. Der Pharmacia Sinica. 2: 105-112.